during contraction. The exchanger is regulated by binding of Ca^{2+} to the intracellular domain. This domain is composed of an α-catenin-like domain (CLD) that connects two structurally homologous Ca^{2+} binding domains (CBD1 and CBD2) to the transmembrane domain of the exchanger. NMR and X-ray crystallographic studies have provided structures for the isolated CBD1 and CBD2 domains and have suggested how Ca^{2+} binding alters their structures and motional dynamics. It remains unknown how Ca^{2+} binding to the intact Ca^{2+} sensor signals the transmembrane domain to regulate exchanger activity. We have used site directed spin labeling to address this question. Conventional EPR experiments have shown that: 1) residues in, or near, the Ca^{2+} binding loops of CBD1 and CBD2 show decreased mobility upon Ca^{2+} binding; and 2) residues in the β-sandwich regions are insensitive to Ca^{2+} binding. Double Electron Electron Resonance (DEER) measurements on doubly labeled constructs revealed that: 1) the structure of the β-sandwich domains of CBD1 and CBD2 are not altered upon Ca^{2+} binding; 2) CBD1 and CBD2 do not lie lengthwise antiparallel in close proximity but rather residues in the distal ends that connect to the CLD are greater than 60 Å apart; and 3) residues nearer to the apex of the Ca^{2+} sensor are in close enough proximity to be measured by DEER and these distances are sensitive to Ca^{2+} binding. These studies support recent SAXS studies by Hilge et al. (PNAS 106:14333-8, 2009) and provide additional insight into a structural rearrangement of the intact Ca^{2+} sensor that may be involved in regulation of Na^{+}/Ca^{2+} exchange.

2065-Pos
Structure of the CDB3 - ankD34 Complex from Site - Directed Spin - Labeling Studies
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The association between the cytoplasmic domain of band 3 (CDB3) and ankyrinR forms a critical link between the lipid bilayer of the erythrocyte membrane and its underlying spectrin cytoskeleton. This interaction is responsible for the remarkable mechanical stability of the erythrocyte membrane that is essential for the durability of the erythrocyte. While the structures of CDB3 [1] and ankD34 (repeats 13-24 from full length ankyrinR) [2] have been determined by X-ray crystallography, the structure of the CDB3-ankD34 complex has not been established. Using distance constraints from site-directed spin labeling (SDSL) and DEER spectroscopy, we propose a new structural model of CDB3-ankD34 complex modeled assuming rigid-body docking between the two proteins combined with rigorous modeling of the spin label. Unexpectedly, the new model generated by Rosetta docking calculations and filtered through multiple DEER distance constraints shows features which are quite different from the previously proposed docking model. The binding interface of CDB3 is widely scattered over its peripheral surface but the β6-β7 hairpin loop makes no direct contact with ankD34. Second, the binding interface of ankD34 resides on the opposite side of β-hairpin loops from the concave groove. The validity of our current model is also supported by a series of SDSL and cross-linking experiments where the binding interface of ankD34 was mapped by the model-guided scanning of a series of surface sites on ankD34. Supported by NIH P01 GM080513.
[1] D. Zhang et al., *Blood*, 96, 2925 (2000)
[2] P. Michaely et al., *EMBO J.*, 21, 6387 (2002)

2066-Pos
Structural Origins of Nitroxide EPR Spectra in a β-Barrel Membrane Protein
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Site-directed spin labeling is a powerful tool for studying structure and dynamics in proteins, due to its ability to bypass several fundamental limitations suffered by methodologies such as NMR and x-ray crystallography. The utility of this technique, however, hinges on our ability to reliably interpret EPR lineshapes of spin-labeled proteins, so that spectral features may be unambiguously associated with their structural origins. In the present work, X-ray crystallography has been combined with mutagenesis and a quantitative analysis of EPR spectra to examine for the first time the origins of spectra from a β-barrel membrane protein, BtuB. The hydrocarbon-exposed residue T156C was spin-labeled and gave rise to a two-component EPR spectrum, corresponding to two conformers of the spin-labeled side chain. Quantitative lineshape analysis revealed a dominant population of highly (spatially) ordered yet mobile nitroxide, and a second population of weakly ordered yet immobile nitroxide. EPR spectra show that single mutations to nearest-neighbor residues affect the ordering and or equilibrium of label rotamers, however these changes are small in each case. In the 2.6Å crystal structure of spin-labeled BtuB, the likely source of weak pairwise interaction with nearest-neighbors is attributed to the extent of barrel curvature, β-strand twist, and direction of strand tilt. It is postulated that residues Q158, L160 (periplasmic loop), V166, and L168 (hydrogen-bonded neighbor) may cooperatively stabilize the nitroxide spin label by forming a hydrophobic pocket. This approach is being applied to an additional hydrocarbon-exposed site on BtuB which exhibits a different degree of strand tilt and twist.

2067-Pos
Characterization of the L511P and D512G Mutations in the MsbA Lipid Flippase
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MsbA is a 65kDa lipid flippase found in the inner membrane of Gram-negative bacteria such as E. coli and S. typhimurium. As a member of the ABC transporter superfamily, MsbA contains two nucleotide binding domains and two transmembrane domains, one from each of its two monomers. ABC transporters transport a diverse group of substrates from lipids to antibiotics and their dysfunction contributes to a number of human pathologies including cystic fibrosis. As an essential protein in E. coli, the deletion or dysfunction of MsbA results in the toxic accumulation of lipid A in the inner membrane resulting in membrane instability and cell death. The L511P and D512G mutations have been previously identified through mutational analysis as dysfunctional nucleotide binding domain mutations specific to MsbA and were suggested to have a lower affinity for ATP. To further understand the cause of dysfunction in these point mutations, in vivo growth assays, in vitro ATPase activity assays, DEER and CW EPR spectroscopy studies throughout the ATP hydrolysis cycle were conducted. L511P and D512G were each paired with nine different reporter residues, each in or near an important conserved nucleotide binding domain motif and compared to the reporter residues alone. To identify the stage in the ATP hydrolysis cycle in which the L511P and D512G mutations are dysfunctional, the local tertiary interactions before, during, and after ATP hydrolysis were monitored by EPR spectroscopy at each stage of the ATP hydrolysis cycle.

2068-Pos
Free Radical Generation and Electron Flux in Mitochondrial Fe-S Centers During Cardiac Injury; Changes with Mitochondrial Protective Drug Ranolazine
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Some TCA cycle enzymes, like aconitase, are more susceptible to ischemia reperfusion (IR) injury. Ranolazine (RAN) is cardioprotective against IR injury. It is a late Na^{+} current blocker that may also limit lipid peroxidation and complex I activity. It is unknown if RAN alters the redox state of Fe-S clusters or free radical generation (FRG) to underlie its protection. Here we examined how IR injury affects FRG and Fe-S clusters of aconitase and succinate dehydrogenase, using electron paramagnetic resonance (EPR), and if RAN alters these effects. Guinea pig hearts (n 8) were isolated and perfused with Krebs Ringer buffer and exposed to: a) control, b) 30 min global ischemia, c) 10 μM RAN for 10 min just before ischemia, or d) ischemia and 10 min reperfusion. Hearts were immediately ground in liquid N_2 and packed into EPR tubes. We examined changes in signal intensity in liquid He (10°K) of assigned g 2.016 (aconitase 3Fe-4S), g 1.93 (succinate dehydrogenase 2Fe-2S), g 2.006 (free radical), and g 6.0 (Fe group of cytochrome c). Versus time control (100%), the signal for aconitase Fe-S at the end of ischemia was 46%, suggesting oxidative damage; this was partially restored by 10 min reperfusion to 91% and after I+RAN treatment to 55% of control. Signal intensity for succinate dehydrogenase was unaltered by IR or RAN+IR. The presumptive ubisemiquinone radical signal increased 19% after ischemia, suggesting increased FRG, but only by 4% at 10 min reperfusion. I+RAN treatment decreased the signal by 19%. The signal for cytochrome c (g 6.0) increased 730% after IR, but was only 81% after I+RAN. These data suggest that RAN treatment partially restores electron flow through some Fe-S centers and reduces FRG, which may partially underlie its cardioprotective effects.

2069-Pos
Structural Analysis of the Membrane Docking Geometry of PI(3,4,5)P3-Specific GRP1-PH Domain Via Site-Directed Spin Labeling
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Peripheral membrane binding proteins play critical roles in dynamic cell signaling processes that occur at membrane surfaces. Many of these signaling proteins contain membrane targeting domains that act to mediate signal dependent membrane localization for proper enzyme function. Phosphoinositide-specific

pleckstrin homology (PH) domains are an important class of membrane targeting domains that specifically bind target phosphoinositides present at the surface of inner cell membranes. Aside from target lipid headgrouprecognition, the other protein-lipid interactions that occurduring membrane docking are not well defined. Currently, high-resolution structural characterization of protein-membrane interfaces is difficult to achieve while this information is crucial to a physical chemical understanding of reversible protein-membrane binding. In this study, site-directed spin-labeling and electron paramagnetic resonance (EPR) power saturation measurements were employed to determine membrane depth parameters for the PI(3,4,5)P3-specific GRP1-PH domain docked to synthetic bilayer membranes. A library of nitroxide spin-labeled PH domain mutants was generated using site-directed cysteine mutagenesis and disulfide coupling to a methanethiosulfonate spin label (MTSSL). Subsequently, membrane depth parameters were determined for each spin-labeled position in the membrane-docked state. The depth parameters were then used as constraints to model the angular orientation and depth of penetration that describes the membrane docking geometry. Our preliminary structural model identifies the membrane binding surface of GRP1-PH and characterizes its partitioning into the membrane bilayer. Ultimately, the results of this study will aid in understanding the molecular determinants of the electrostatic search mechanism this PH domain uses to rapidly find its rare target lipid on the plasma membrane surface. Supported by NIH GM063235 (J.J.F.).

2070-Pos

Sequence-Specific Stereomeric Environment in a DNA Duplex Revealed by a Nucleotide-Independent Nitroxide Probe

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In site-directed spin labeling, structural and dynamic information on a parent macromolecule is obtained by monitoring a covalently linked nitroxide radical using electron paramagnetic resonance (EPR) spectroscopy. Our group have developed a method of attaching nitroxide species, such as 1-oxyl-4-bromo-2,2,5,5-tetramethylpyrroline (R5a), to a specific nucleotide position within a target DNA or RNA sequence. The method relies on site-specific introduction of a phosphorothiate during the solid phase chemical synthesis of nucleic acids, and at each given labeling site the nitroxide is attached to one of two phosphorothioate diastereomers (Rp or Sp) in an approximately 50/50 ratio. We have recently reported that variations in DNA structural and dynamic features at the level of an individual nucleotide can be detected using R5a attached to mixed phosphorothioate diastereomers, in which an observed EPR spectrum is presumably a sum of those obtained from either diastereomer (Popova et al., Biochemistry, 2009, 48, 8540-50). In this work, we report X-band EPR spectra of R5a attached to purified Rp and Sp diastereomers at different sites within a B-form DNA duplex. Results are compared to those obtained with mixed nitroxide diastereomers, and advantages and limitations are discussed regarding the necessity of diastereomer separation when probing DNA local environment. Our work is a further step forward in developing a SDSL methodology that may provide a mean for studying structure and dynamics in large DNA molecules.

Nano-Materials

2071-Pos

Analysis of Postphotoactivation Scanning Diffusion Profiles for Multiple Species with Distributed Diffusion Coefficients

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Postphotoactivation scanning (PPS) is a method for quantifying diffusion coefficients of particles in simple and complex materials (e.g., hydrogels) over length scales ~100μm-mm (Geonnotti et al., 2008). Diffusing particles are labeled with caged fluorophore, and a slit-shaped region of sample is exposed to UV to generate a line of fluorescence. A high-resolution scanner quantifies intensity profiles as particles diffuse out from the fluorescent region over time; these are fit to the solution of the diffusion equation to obtain the diffusion coefficient. We use this technique to measure mobility of HIV-like liposomes. Here, we describe a novel method for analyzing PPS profiles for multiple diffusing species with a distribution (α) of diffusion coefficients (D). To determine $\alpha(D)$, we generated sets of diffusion profiles for a discretized range of D by numerically solving the diffusion equation using the experimental initial condition and assuming given D. We computed net diffusion profiles resulting from the sums of profiles with distribution α. We used an optimization scheme to deduce $\alpha(D)$ that minimized the squared difference of observed and computed profiles. The method was validated using simulated and experimental data. Experimental results for fluorescein diffusing in PBS (D=4.3×10^{-6} cm^2/s) are similar to literature values. We also measured diffusion coefficients of solutions of labeled ~100nm liposomes (D=3.2 ± 2×10^{-8} cm^2/s, n=4). We were able to resolve 2 distinct peaks in α corresponding to the D of liposomes and of free label. Measured values for diffusion coefficients of liposomes are similar to those predicted by Stokes-Einstein (D=3.9 ± 1×10^{-8} cm^2/s). We are using the technique to analyze interactions of HIV-like liposomes and anti-HIV antibodies. The method can be applied to describe diffusion of multiple species within complex materials. [Supported by Duke CFAR and NIH AI48103]

2072-Pos

Mucus Rheological Properties Altered by Functional Nanoparticles

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Multi-functionalized nanoparticles (NPs) have recently been extensively explored for their potential in novel drug delivery and nanomedicine applications. Functionalized NPs based local drug delivery across the mucosal epithelia has gained much interest.

Despite reports confirming cellular nanotoxicity effects, possible health hazards resulted from mucus rheological disturbances induced by NPs are underexplored.

Accumulation of viscous, poorly dispersed and less transportable mucus that could result in improper mucus rheology and dysfunctional mucociliary clearance are typically found to associate with many respiratory diseases such as asthma, cystic fibrosis (CF) and COPD (chronic obstructive pulmonary disease). Whether functionalized NPs can alter mucus rheology and its operational mechanisms are not resolved. Here we show for the first time that positively-charged functionalized NPs can effectively induce mucin aggregation and hinder mucin gel hydration. These NPs significantly increase the size of aggregated mucin approximately 30 times within 24 hrs. EGTA (ethylene glycol tetraacetic acid, 2 mM) chelation of indigenous mucin crosslinkers (Ca^{2+} ions) was unable to effectively disperse NPs-induced aggregated mucins. We also found that positively-charged NPs can significantly reduce the swelling kinetics and hydration of newly released mucus. Our results have demonstrated that positively charged functionalized NPs can serve as effective crosslinkers hindering mucin disaggregation and dispersion resulting in potential dysfunctional mucociliary clearance and health problems. This report also highlights the unexpected health risk of NP-induced change in mucus rheology and possible mucociliary transport impairment on epithelial mucosa. In addition, our data can serve as a prospective guideline for designing nanocarriers specific for mucosal epithelia drug delivery applications.

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2073-Pos

Silica Nanoparticles Permeabilize Lipid Bilayers

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Potential toxic effects of synthetic nanoparticles are of great public concern. Presently, the impact of nanoparticles at a cellular level is assessed by adding nanoparticles to cell cultures and subsequent evaluation of particle uptake by confocal fluorescence microscopy and of cell viability by conventional (fluorescence) assays. Cytotoxic effects of nanoparticles have been reported but a correlation between nanoparticle properties (e.g. size, shape, surface chemistry) and cell viability remains elusive. However, cellular uptake of nanoparticles is almost universally observed. Membrane translocation of nanoparticles is generally considered to be an active process, requiring the presence of receptors that mediate encapsulation of the nanoparticles into an intracellular vesicle, from which the particles may or may not escape into the cytosol.

Using electrophysiological methods we have demonstrated that spherical silica nanoparticles, under development for intracellular drug delivery, are able to permeabilize protein-free lipid bilayers as a function of size and surface charge. Single channel-like conductances, similar to those induced by membrane-disrupting β-amyloid peptides, are observed for rigid sterol-containing bilayers. For more fluid bilayers of DOPC the conductance gradually increases until the bilayer disintegrates, which has also been observed for cytotoxic amyloid oligomers. The most disruptive nanospheres were shown by confocal fluorescence microscopy to accumulate at the bilayer surface, and we demonstrated that a fraction of these particles translocate across the lipid bilayer, suggesting that passive uptake of nanoparticles may contribute to cellular uptake.